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(54) Title: CONTROLLED RELEASE GROWTH HORMONE CONTAINING MICROSPHERES

(57) Abstract

Growth hormone polymeric controlled release systems are described wherein the growth hormone retains good biological activity and is released over an extended period of time following administration by injection. In the preferred embodiment, human growth hormone polymeric microspheres are made using very cold temperatures to freeze the polymer-growth hormone mixtures into polymeric microspheres with very high retention of biological activity and material. Sustained release of biologically active growth hormone is achieved when the microspheres are tested *in vitro*, extending over a period of greater than one day to several months. Altered release can be achieved by inclusion of degradation modifiers, pore forming agents, and stabilizers of the growth hormone.

INSIDE FIG. 1

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CONTROLLED RELEASE GROWTH HORMONE CONTAINING MICROSPHERES

Background of th Invention

This invention generally relates to polymeric microspheres for controlled release of growth hormone.

Human Growth hormone (hGH) is a protein secreted by the pituitary gland. The protein contains 191 amino acids and has a molecular weight of 22,000. hGH causes growth of all tissues of the body which are capable of growth. In addition, to its general effect in causing growth, the hormone is involved in the following metabolic effects: 1) increased cellular protein synthesis, 2) decreased glucose utilization, and 3) increased mobilization of fatty acids from adipose tissue. hGH is used to treat hypopituitary dwarfism and is administered three times per week by the subcutaneous or intramuscular route.

The administration of growth hormone generally necessitates frequent intramuscular (IM) or subcutaneous (SQ) injections. The advantages of a controlled release formulation for growth hormone include increased patient compliance and acceptance by reducing the number of injections, increased therapeutic benefit by eliminating the peak and valley changes in blood levels, and potentially lowering the total administered amount of drug by reducing peaks and valleys.

One means for controlling blood levels of a compound is to administer it in the form of a polymeric matrix that releases compound as a function of polymer degradation and/or drug diffusion. A variety of biodegradable and non-biodegradable polymers have been used for such applications, including polyesters such as poly(lactide-co-glycolide)s, polyanhydrides, polyorthoesters, and ethylenevinyl acetate polymers. In general, release is controlled by selection of the appropriate polymer, encapsulation conditions, and drug loading and excipients.

Examples of these polymeric systems are described in U.S. Patent Nos. 4,891,225 to Langer and 4,906,474 to Langer (polyanhydrides), 4,391,797 to Folkman, et al., (ethylenevinyl acetate polymers), 4,767,628 to Hutchinson (polylactide, polylactide-co-glycolide acid), and 4,530,840 to Tice, et al. (polylactide, polyglycolide, and copolymers).

However, controlled release at the desired rate and over the desired period is difficult to achieve. Moreover, the conditions used to encapsulate the drug must not result in degradation of the drug to be delivered nor must the drug react with the polymeric matrix so as to inactivate or bind the drug. As important in a clinical situation, the delivery means must be cost effective to produce, stable to storage, and administrable using standard methodology.

It is therefore an object of the present invention to provide a method for making microspheres containing growth hormone with very little loss of activity or material, especially human growth hormone.

It is a further object of the present invention to provide a method for making microspheres formed from a broad range of polymers which contain active growth hormone releasable in a controlled fashion, and the microspheres produced by such a process.

Summary of the Invention

Growth hormone polymeric controlled release systems are described wherein the growth hormone retains good biological activity and is released over an extended period of time following administration. In the preferred embodiment, the growth hormone polymeric microspheres are made using very cold temperatures to freeze the polymer-growth hormone mixtures into polymeric microspheres with very high retention of biological activity and material. Polymer, preferably a poly(lactide), is dissolved in a solvent such as

methylene chloride together with powdered growth hormone. The polymer/growth hormone mixture is atomized into a vessel containing a frozen non-solvent such as ethanol, overlayed with a liquified gas such as nitrogen, at a temperature below the freezing point of the polymer/active agent solution or suspension. The atomized particles freeze into microspheres upon contacting the cold liquified gas, then sink onto the frozen non-solvent layer. The frozen non-solvent is then thawed. As the non-solvent thaws, the microspheres are still frozen and sink into the liquid non-solvent. The solvent in the microspheres also thaws and is slowly extracted into the non-solvent, resulting in hardened microspheres containing the growth hormone.

Examples using human growth hormone show sustained release of biologically active growth hormone when the microspheres are tested *in vitro* or *in vivo*, extending over a period of one day up to three months. Altered release can be achieved by inclusion of polymer degradation modifiers, pore forming agents, and stabilizers of the growth hormone.

Brief Description of the Drawings

Figure 1 is a graph of the average change in weight (g) over 14 days for rats administered 80 μg growth hormone daily (dark circles), 20 μg growth hormone daily (open squares), and growth hormone released from microspheres (dark squares) over time (days).

Detailed Description of the Invention

Growth hormone containing microspheres are made by incorporating the growth hormone into a biocompatible polymeric microsphere, up to approximately 50% w/w, wherein the microsphere containing the growth hormone is characterized by sustained controlled release of the growth hormone over a period of at least 24 hours up to

a period of one to three months. In the preferred embodiment, the polymer is biodegradable, most preferably by hydrolysis, the microspheres have a diameter of less than one hundred eighty microns, most preferably less than seventy microns, and are suitable for administration by injection subcutaneously or intramuscularly (a size suitable for injection through a 23-gauge needle would be less than 180 μm in diameter), and the microspheres contain from 0.01% by weight up to approximately 50% by weight human growth hormone.

As used herein, "microsphere" is used to mean solid spheres formed of polymer having growth hormone dispersed throughout, as well as microparticulates and microcapsules, unless otherwise noted. Microparticulates are specifically referred to when describing irregularly shaped polymer or polymer-drug particles. Microcapsules are spherical shaped polymer devices having a non-polymer core or a core of a different polymer than the outer shell.

As used herein, "sustained" or "extended" release of the growth hormone can be continuous or discontinuous, linear or non-linear. This can be accomplished using one or more types of polymer compositions, drug loadings, selections of excipients or degradation enhancers, or other modifications, administered alone, in combination or sequentially to produce the desired effect.

Growth hormone is available in the form of a lyophilized powder containing physiological buffers and salts.

Methods for incorporation of Growth hormone into microspheres.

A variety of techniques are known by which active agents can be incorporated into polymeric microspheres.

Spray Drying

In spray drying, the polymer and growth hormone are mixed together in a solvent for the polymer, then

the solvent is evaporated by spraying the solution, leaving polymeric droplets containing the active agent. Spray drying is reviewed in detail by K. Masters in "Spray Drying Handbook" (John Wiley & Sons, New York 1984); and Patrick B. Deasy in "Microencapsulation and Related Drug Processes" (Marcel Dekker, Inc., New York 1984), the teachings of which are incorporated herein. Spray drying is not preferred since it may result in some loss of activity due to the heat generated in the process as well as in loss of considerable amounts of the material due to sticking of the polymer to the large surface area on the sides of the chamber.

Solvent Evaporation

Solvent evaporation techniques can be used to form microspheres. These techniques involve dissolving the polymer in an organic solvent which contains either dissolved or dispersed active agent. The polymer/active agent solution is then added to an agitated continuous phase which is usually aqueous. Emulsifiers are included in the aqueous phase to stabilize the oil-in-water emulsion. The organic solvent is then evaporated over a period of several hours or more, thereby depositing the polymer around the core material. Solvent can be removed from the microspheres in a single step, as described in U.S. Patent No. 3,737,337 and U.S. Patent No. 3,523,906, or in U.S. Patent No. 3,691,090 (under reduced pressure), or by the application of heat, as shown in U.S. Patent No. 3,891,570. A two-step technique is described in U.S. Patent No. 4,389,330. Freeze drying has also been used to remove the solvent from microspheres, as reported by Sato, et al, in "Porous Biodegradable Microspheres for Controlled Drug Delivery. I. Assessment of Processing Conditions and Solvent Removal Techniques," Pharmaceutical Research 5, 21-30 (1988). The teachings of these methods are incorporated herein.

Solvent evaporation works reasonably well but is not preferred since the amount of incorporated material is usually lower than the theoretical values due to loss of drug to the aqueous phase, as reported by Benita, et al., in "Characterization of Drug Loaded Poly(d,l-lactide) Microspheres," J. Pharm. Sci. 73, 1721-1724 (1984).

Phase separation

Phase separation techniques can also be used to form microspheres. These techniques involve the formation of a water-in-oil emulsion or oil in water emulsion. The polymer is precipitated from the continuous phase onto the active agent by a change in temperature, pH, ionic strength or the addition of precipitants. For example, U.S. Patent No. 4,675,800, et al., describes the formation of poly(lactic-co-glycolic) acid microspheres containing active proteins. The protein is first dissolved in the aqueous phase of a water-in-oil emulsion or dispersed as a solid in the polymer phase. Polymer is then precipitated around the aqueous droplets or drug particles by addition of a non-solvent for the polymer such as silicone oil. The final product, as with most phase separation techniques, is in the form of a microcapsule. Microcapsules contain a core material surrounded by a polymer membrane capsule. Microcapsules are not the preferred embodiment for delivery of growth hormone, however, since the release kinetics of active agents from these devices can be difficult to control.

Although these phase separation techniques result in the formation of microspheres containing active agents, active agent is often lost during the solvent extraction process. In addition, as with spray drying, biologically active proteins may be denatured during the process.

Rapid freezing, solvent extraction

The preferred method for making growth hormone microspheres having the desired characteristics is described in U.S. Patent No. 5,019,400 to Gombotz, et al, the teachings of which are incorporated herein.

There are two principal embodiments of the system for making microspheres: a combination liquified gas - frozen non-solvent system and a frozen non-solvent system.

Polymer and agent to be encapsulated in solution are atomized using an ultrasonic device into a liquified gas. The atomized particles freeze when they contact the liquified gas (liquid nitrogen), forming frozen spheres. These sink to the surface of the frozen non-solvent (ethanol). The liquid gas is evaporated and the spheres begin to sink into the non-solvent as the non-solvent thaws. The solvent in the spheres is extracted into the non-solvent to form microspheres containing the agent to be encapsulated. Other non-solvents such as hexane are added to the non-solvent (ethanol) to increase the rate of solvent extraction from certain polymers, where appropriate, for example, when spheres are formed of polylactide-co-glycolide polymers.

The liquified gas can be liquid argon (-185.6°C), liquid nitrogen (-195.8°C), liquid oxygen (-182.9°C) or any other gas that results in the immediate freezing of the atomized particles into frozen spheres. Oxygen is not preferred since it is explosive and may cause oxidation of the protein.

Alternatively, a cold non-solvent for the polymer can be substituted for the combination of liquified gas-frozen no-solvent, provided the temperature of the non-solvent is below the freezing temperature of the polymer/active agent solution.

In both embodiments, it is important that the polymer/active agent freeze immediately upon contacting

the cold liquid, and then be slowly thawed and the polymer solvent extracted from the microspheres.

The thawing rate is dependent on the choice of solvents and non-solvents. It is important to select a solvent for the polymer having a higher melting point than the non-solvent for the polymer so that the non-solvent melts first, allowing the frozen microspheres to sink into the liquid where they later thaw. If a cold liquid non-solvent system for making the polymeric microspheres is used, the microspheres will sink immediately into the non-solvent. As the solvent in the microsphere thaws, it is extracted into the non-solvent. The solvent for the polymer and the non-solvent for the polymer must be miscible to allow extraction of the solvent from the microspheres. Table 1 shows some polymer/solvent/non-solvent systems that can be used in this process along with their melting points.

Table 1: Polymers and Appropriate Solvents and Non-Solvents Systems, with Solvent and Non-Solvent Melting Points °C

POLYMER	SOLVENT	NON-SOLVENT
Poly(lactide	Methylene Chloride (-95.1)	Ethanol (-114.5)
	Chloroform (-63.5)	Methanol (-97.5)
Poly(lactide-co-glycolide acid)	Ethyl Acetate (-83.6)	Ethanol (-114.5)
	Acetone (-95.4)	Ethyl ether (-116.3)
	Methylene Chloride (-95.1)	Isopentane (-130)
Poly(caprolactone)	Methylene Chloride (-95.1)	Ethanol (-114.5)
Poly (vinyl alcohol)	Water (0)	Acetone (-95.4)
Ethylene-vinyl acetate	Methylene Chloride (-95.0)	Ethanol (-114.5)

The polymer/active agent/solvent mixture can be sprayed into the cold liquid, either the liquified gas or the cold non-solvent, using a variety of devices which can be used to form small particles, including sonic nozzles, pressure nozzles, pneumatic nozzles and rotary atomizers.

A wide range of sizes of microspheres can be made by varying the droplet size, for example, by changing the nozzle diameter. If very large spheres are desired, the spheres can be extruded through a syringe directly into the cold liquid. Increasing the inherent viscosity of the polymer solution can also result in an increasing microspheres size. The size of the spheres produced by this process can range from greater than 1000 to 5 microns in diameter. A preferred size range for injectable microspheres is from 30 to 180 microns in diameter. The microspheres made by this technique are spherical in shape.

Selection of the Polymeric Matrix

Polymers that can be used to form the microspheres include bioerodible polymers such as poly(lactide), poly(lactide-co-glycolide), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates and degradable polyurethanes, and non-erodible polymers such as polyacrylates, ethylene-vinyl acetate polymers and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, and polyethylene oxide. Almost any type of polymer can be used provided the appropriate solvent and non-solvent are found which have the desired melting points. In general, a polymer solution is prepared containing between 1% polymer and 30% polymer, preferably 5-10% polymer.

In the preferred embodiment, a poly(lactide) is used. As used herein, this term includes polymers of lactic acid or lactide alone, copolymers of lactic acid and glycolic acid, copolymers of lactide and glycolide, mixtures of such polymers and copolymers, the lactic acid or lactide being either in racemic or optically pure form. It is most desirable to use polylactides in the range of molecular weight up to 100,000.

The release of the growth hormone from these polymeric systems can occur by two different mechanisms. The drug can be released by diffusion through aqueous filled channels generated in the dosage form by the dissolution of the drug or by voids created by the removal of the polymer solvent during the original microencapsulation. The second mechanism is enhanced release due to the degradation of the polymer. With time the polymer begins to erode and generates increased porosity and microstructure within the device. This creates additional pathways for drug release.

The degradation of the polymers occurs by spontaneous hydrolysis of the ester linkages on the backbone. Thus the rate can be controlled by changing polymer properties influencing water uptake. These include the monomer ratio (lactide to glycolide), the use of L-Lactide as opposed to D/L Lactide, and the polymer molecular weight. These factors determine the hydrophilicity and crystallinity which ultimately govern the rate of water penetration. Hydrophilic excipients such as salts, carbohydrates and surfactants can also be incorporated to increase water penetration into the devices and thus accelerate the erosion of the polymer.

By altering the properties of the polymer and the properties of the dosage form, one can control the contribution of each of these release mechanisms and alter the release rate of growth hormone. Slowly eroding polymers such as poly L-lactide or high molecular weight poly(lactide-co-glycolide) with low

glycolide compositions will cause the release to become diffusion controlled. Increasing the glycolide composition and decreasing the molecular weight enhances both water uptake and the hydrolysis of the polymer and adds an erosion component to the release kinetics.

The release rate can also be controlled by varying the loading of growth hormone within the microspheres. Increasing the loading will increase the network of interconnecting channels formed upon the dissolution of the drug and enhance the release of drug from the microspheres. The preferred range of growth hormone loadings is in the range of 3-30% (w/w).

Polymer hydrolysis is accelerated at acidic or basic pH's and thus the inclusion of acidic or basic excipients can be used to modulate the polymer erosion rate. The excipients can be added as particulates, can be mixed with the incorporated growth hormone or can be dissolved within the polymer.

Excipients can be also added to the growth hormone to maintain its potency depending on the duration of release. Stabilizers include carbohydrates, amino acids, fatty acids, and surfactants and are known to those skilled in the art. In addition, excipients which modify the solubility of growth hormone such as salts, complexing agents (albumin, protamine) can be used to control the release rate of the protein from the microspheres.

Additives to alter release rate, degradation rate, stability of growth hormone

Stabilizers for the growth hormone are based on ratio to the protein on a weight basis. Examples include carbohydrate such as sucrose, lactose, mannitol, dextran, and heparin, proteins such as albumin and protamine, amino acids such as arginine, glycine, and threonine, surfactants such as Tween™ and Pluronic™, salts such as calcium chloride and sodium phosphate, and

lipids such as fatty acids, phospholipids, and bile salts.

The ratios are generally 1:10 to 4:1, carbohydrate to protein, amino acids to protein, protein stabilizer to protein, and salts to protein; 1:1000 to 1:20, surfactant to protein; and 1:20 to 4:1, lipids to protein.

Degradation enhancers are based on weight relative to the polymer weight. They can be added to the protein phase, added as a separate phase (i.e., as particulates) or can be codissolved in the polymer phase depending on the compound. In all cases the amount should be between 0.1 and thirty percent (w/w, polymer). Types of degradation enhancers include inorganic acids such as ammonium sulfate and ammonium chloride, organic acids such as citric acid, benzoic acids, heparin, and ascorbic acid, inorganic bases such as sodium carbonate, potassium carbonate, calcium carbonate, zinc carbonate, and zinc hydroxide, and organic bases such as protamine sulfate, spermine, choline, ethanolamine, diethanolamine, and triethanolamine and surfactants such as Tween™ and Pluronic™.

Pore forming agents to add microstructure to the matrices (i.e., water soluble compounds such as inorganic salts and sugars). They are added as particulates. The range should be between one and thirty percent (w/w, polymer).

Administration of the microspheres to a patient.

An effective amount of the microspheres containing growth hormone are administered to a patient by injection subcutaneously, intramuscularly, intraperitoneally, and intradermally, by administration to mucosal membranes (such as intranasally or by means of a suppository), or by *in situ* delivery to provide the desired dosage of growth hormone, based on the known parameters for treatment with growth hormone of the various medical conditions.

The present invention is further described by the following non-limiting examples.

Example 1: Preparation of poly(L-lactic acid) microspheres containing growth hormone.

0.54 of poly D/L lactide co-glycolide 50:50 (Inherent viscosity 0.16) (Birmingham Polymers, Birmingham AL) was dissolved in 3.2 ml of methylene chloride. To this polymer solution was added 60 mg of lyophilized human growth hormone containing zinc ions at a 4:1 molar ratio and sodium bicarbonate (10 mg). The lyophilized protein had particle sizes in the 2-5 micron range. The solution was placed in 10 ml gas tight syringe. A 200 ml amount of 100% ethanol was added to round polypropylene container (17 cm diameter, 8 cm deep). This solution was frozen in liquid nitrogen and covered with 500 ml of liquid nitrogen. The polymer protein mixture was pumped from the syringe via a syringe pump at 2 ml/min, into an ultrasonic nozzle (Mode, Sonics and Material, Danbury CT) that was placed above the container of liquid nitrogen and frozen ethanol. The nozzle atomized the suspension into droplets which froze upon contact with the liquid nitrogen and formed microspheres which sank onto the frozen ethanol.

The container was placed at -80°C where the liquid nitrogen evaporated and the ethanol melted with time. As the ethanol thaws, the microspheres settle into the liquid where the methylene chloride is extracted. After 24 hours, an additional 200 ml of 100% ethanol prechilled to -80°C was added to the container. After three days, the slurry of microspheres and ethanol was filtered using a 1 micron Durapore membrane (Millipore, Bedford, MA). The filtered microspheres were then lyophilized.

Example 2: ***In vivo*** **assay of growth hormone released from poly(D/L-lactide co-glycolide) microspheres.**

Microspheres as produced in example 1 were tested in the rat bioassay detailed in the British Pharmacopeia. Hypophysectomized rats were obtained from Taconics, Germantown, New York. Three groups of animals were utilized. Groups 1 and 2 received a single daily injection of hGH of 20 and 80 μg respectively. Group 3 received 18 mg of microspheres injected subcutaneously in the dorsum. The animals were fed with a standard diet and allowed free access to water.

The increase in body weight is shown in Figure 1. The injectable formulation provides sustained release of potent hormone which results in body weight gain.

We claim:

1. A polymeric microsphere having a diameter of less than 1000 microns, formed of a biocompatible polymer selected from the group consisting of poly(lactide), poly(lactide-co-glycolide)s, poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates, degradable polyurethanes, polyacrylates, polymers of ethylene-vinyl acetate and other acyl substituted cellulose acetates and derivatives thereof, polysaccharides, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, copolymers and mixtures thereof, containing growth hormone dispersed through the polymer in a concentration of between 0.1% and 50% by weight, and an excipient selected from the group consisting of excipients modulating polymer erosion rate, excipients stabilizing human growth hormone potency, and excipients modifying the solubility of growth hormone, and releasing the growth hormone under physiological conditions over a period of time greater than one day.

2. The microspheres of claim 1 wherein the polymer is a polylactide or poly(lactide-co-glycolide).

3. The microspheres of claim 1 wherein the diameter is less than 180 microns.

4. The microspheres of claim 1 wherein the erosion rate modulating agent is a pore forming agent added to the polymer in particulate form in a concentration of between one and thirty percent (w/w, polymer).

5. The microspheres of claim 1 wherein the stabilizers are selected from the group consisting of carbohydrates, amino acids, proteins, lipids, salts, fatty acids, and surfactants.

6. The microspheres of claim 1 wherein the excipients which modify the solubility of growth hormone are present in a concentration of between 0.1 and thirty percent (w/w, polymer) and are selected from the group consisting of salts, complexing agents, inorganic acids, organic acids, inorganic bases, organic bases, and surfactants.

7. A method for administering growth hormone comprising administering a biocompatible polymeric microsphere containing between 0.1 and 50% growth hormone and having a diameter of less than one hundred eighty microns, formed of a biocompatible polymer selected from the group consisting of poly(lactide), poly(lactide-co-glycolide)s, poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates, degradable polyurethanes, polyacrylates, polymers of ethylene-vinyl acetate and other acyl substituted cellulose acetates and derivatives thereof, polysaccharides, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, copolymers and mixtures thereof, containing growth hormone dispersed through the polymer in a concentration of between 0.1% and 50% by weight and an excipient selected from the group consisting of excipients modulating polymer erosion rate, excipients stabilizing human growth hormone potency, and excipients modifying the solubility of growth hormone, into a patient in need of treatment with growth hormone, wherein the growth hormone is released over a period of time in excess of one day.

8. The method of claim 7 formed of a biocompatible polymer selected from the group consisting of poly(lactide), poly(lactide-co-glycolide)s, poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates, degradable polyurethanes, polyacrylates, polymers of ethylene-vinyl acetate and other acyl substituted cellulose acetates and derivatives thereof, polysaccharide, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, and copolymers and mixtures thereof.

9. The method of claim 8 wherein the polymer is a polylactide or poly(lactide-co-glycolide).

10. The method of claim 7 wherein the diameter of the microspheres is less than 70 microns.

11. The method of claim 7 wherein the microspheres are administered by injection intramuscularly, subcutaneously, intraperitoneally, or intradermally.

12. The method of claim 7 wherein the microspheres are administered by application to a mucosal membrane.

13. A method for making a device for controlled sustained administration of growth hormone comprising

making polymeric microspheres having a diameter of less than one hundred eighty microns, formed of a biocompatible polymer selected from the group consisting of poly(lactide), poly(lactide-co-glycolide), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates, degradable polyurethanes, polyacrylates, polymers of ethylene-vinyl acetate and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride,

poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, and copolymers and mixtures thereof containing growth hormone in a concentration of between 0.1% and 50% by weight, wherein the microspheres further comprise an excipient selected from the group consisting of excipients modulating polymer erosion rate, excipients stabilizing Growth hormone potency, and excipients modifying the solubility of growth hormone, by

a) freezing droplets of polymer-growth hormone solution by atomizing the droplets into a liquified gas, having a temperature below the freezing point of the polymer solution effective to immediately freeze the atomized polymer solution upon contact, said liquified gas overlaying a layer of frozen liquid non-solvent for the polymer, wherein the polymer solvent is miscible in the liquid non-solvent;

b) thawing the polymer solvent in the frozen droplets of polymer solution; and

c) extracting the solvent from the droplets into a liquid non-solvent to form spherical polymeric microspheres.

14. The method of claim 13 wherein the erosion rate modulating agent is a pore forming agent added to the polymer in particulate form in a concentration of between one and thirty percent (w/w, polymer).

15. The method of claim 13 wherein the stabilizers are selected from the group consisting of carbohydrates, amino acids, proteins, lipids, salts, fatty acids, and surfactants.

16. The method of claim 13 wherein the excipients which modify the solubility of growth hormone are present in a concentration of between 0.1 and thirty percent (w/w, polymer) and are selected from the group consisting of salts, complexing agents, inorganic acids, organic acids, inorganic bases, organic bases, and surfactants.

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THIS FIG.

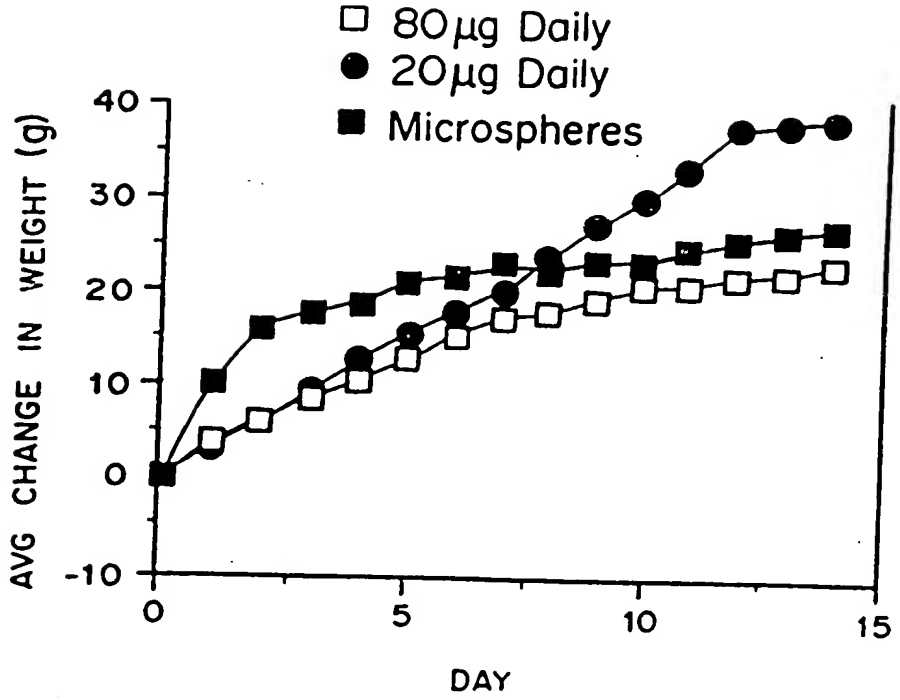

FIG. 1

This 25%

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 93/11621

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K9/16 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 330 180 (BIOMATERIALS UNIVERSE INC.) 30 August 1989	1-12
Y	see claims 1-4 see page 3, line 10 - line 25 see page 3, line 45 - line 56 see page 4, line 46 - page 5, line 6	13-16
Y	WO,A,90 13780 (ENZYTECH INC.) 15 November 1990 see the whole document	1-16
P,Y	WO,A,93 17668 (ALKERMES CONTROLLED THERAPEUTICS INC.) 16 September 1993 see the whole document	1-16

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 1994	03.03.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Ventura Amat, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 93/ 11621

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 7-12 are directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/US 93/11621

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0330180	30-08-89	JP-A-	1216918	30-08-89
		US-A-	5100669	31-03-92
WO-A-9013780	15-11-90	US-A-	5019400	28-05-91
		AU-B-	621751	19-03-92
		AU-A-	5530990	29-11-90
		CA-A-	2030550	02-11-90
		EP-A,B	0424516	02-05-91
		JP-T-	3504389	26-09-91
WO-A-9317668	16-09-93	NONE		